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Transcription factor Sp1 recognizes promoter sequences from the monkey genome that are similar to the simian virus 40 promoter

(RNA polymerase II/*in vitro* transcription/DNase protection assay/host/virus homology)

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ABSTRACT **A 440-base-pair fragment of African green monkey genomic DNA shares homology with the transcriptional regulatory region of simian virus 40 (SV40) and has been reported to direct transcription *in vivo*. We find that two regions within this fragment bind the promoter-specific cellular transcription factor Sp1 and are protected in DNase protection ("footprinting") experiments. As in SV40, binding occurs in regions containing multiple copies of the sequence GGGCGG. These regions, when fused to the proximal, or "TATA box," element of the herpes simplex virus thymidine kinase promoter, are able to direct Sp1-dependent transcription *in vitro*. The finding that Sp1 is capable of productive interaction with sequences taken from a cellular promoter supports the idea that Sp1 may play a role in modulating transcription of cellular genes.**

Sequences required for the control of transcription and replication in the DNA tumor virus SV40 (simian virus 40) reside in a noncoding region located between divergent transcription units. This 25-base-pair (bp) stretch of DNA contains several regulatory elements that partially overlap but are genetically separable (1). One of these is a bidirectional transcriptional promoter, located in a region containing two exact and one degenerate 21-bp tandem sequence repeats. Nested within these repeats are six copies of the hexanucleotide GGGCGG, known as the "GC box." This promoter element is required for transcription of the early and late viral strands both *in vivo* and with an *in vitro* transcription system based on a whole-cell extract (2–11). Previous biochemical studies in our laboratory have identified a cellular factor, Sp1, that binds the DNA in the region of the GC boxes and activates transcription by RNA polymerase II (12–14). We have established that Sp1 is a promoter-specific factor required for SV40 transcription but not for a diverse group of other viral and cellular promoters that were tested.

The presence of Sp1 in uninfected cells strongly suggests that this factor plays a role in cellular as well as viral transcription. The elucidation of this cellular role will be aided by isolation of cellular sequences bearing Sp1 interaction sites. Several good candidates exist among a group of African green monkey genomic clones (15). These DNAs cross-hybridize to a fragment of SV40 DNA containing the origin and transcriptional control elements and appear to be members of an interspersed repetitive sequence family of about 80 members. Three of these cloned fragments have been sequenced and found to contain multiple copies of the GC box, as well as internal direct sequence repeats and homology to the region of the dyad symmetry in the SV40 replication origin (16).

One member of the family, called 7.02, has been characterized extensively and appears to contain a transcriptional promoter (17). Nuclease S1 analysis using probes specific for the 7.02 region, which do not cross-hybridize with other members of the family, detects transcripts proceeding outward from the SV40-like region in both directions on the chromosome. Moreover, two DNase-hypersensitive sites are present, one in the 440-bp 7.02 segment itself, the other in flanking DNA (18). Such sites are often indicative of promoter activity. Finally, linkage of 7.02 sequences to a xanthine phosphoribosyltransferase gene creates a product able to transform cells 3- to 15-fold more efficiently than a promoterless control plasmid (17).

The sequence homology between 7.02 and SV40 and the fact that both regions contained functional bidirectional promoters suggested that the 7.02 segment might contain one or more Sp1 interaction sites. Previously, it was shown that one region of 7.02 is protected by Sp1 from both DNase cleavage and dimethyl sulfate modification (14). We report here that there are two regions within the 7.02 DNA that bind Sp1 and are protected from DNase I in a "footprinting" assay, that the protection is seen on both strands of the DNA, and that 7.02 and SV40 DNA compete with each other for Sp1 binding. More importantly, we find that when Sp1-binding sequences from 7.02 are fused to a truncated herpes simplex virus thymidine kinase promoter lacking its distal control regions, they recreate a functional, Sp1-dependent promoter. Thus, our results show not only that the monkey DNA contains Sp1 binding regions but that these sites can actively direct transcriptional initiation.

MATERIALS AND METHODS

Plasmids. The plasmid p7.02 contains a 440-bp *Ava* I–*Sal* I fragment of monkey DNA inserted between the *Bam*HI and *Sal* I sites of pBR322 (16). The plasmid ptk/Δ5′-32 (19) contains the herpes thymidine kinase structural gene (*tk*) and proximal promoter element, or "TATA box," but is missing the distal promoter elements required for efficient transcription. A *Bam*HI linker has been inserted 32 bp upstream from the transcription start site.

Fusions between the GC boxes of p7.02 and the truncated *tk* promoter are diagrammed in Fig. 4. Three clones were made with the G-rich strand of the GC boxes reading in the sense of the *tk* mRNA. We refer to this as the "SV40 late-like" orientation because in the viral genome the G-rich strand reads in the sense of the late region mRNA. The fusion p363L.tk was made by cutting the monkey DNA with *Sph* I (position 63) and *Nar* I (position 363), inserting the isolated fragment into the *Sph* I and *Acc* I sites of the pUC18 polylinker (20), then inserting the *tk* gene fragment between the *Bam*HI and *Eco*RI sites of the same pUC18 vector. Eight

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Abbreviations: SV40, simian virus 40; tk, thymidine kinase; bp, base pair(s).

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base pairs of pUC polylinker, containing an *Xba* I site, separate the monkey and *tk* segments. Two additional fusion clones, p274L.tk and p254L.tk, were derived from p363L.tk by cleavage at the *Xba* I site and digestion with BAL-31 to nucleotides 274 or 254, respectively. The remnant of the *tk* gene was removed from the plasmid by digestion with *Eco*RI, and a replacement *tk* gene was inserted as a *Bam*HI–*Eco*RI fragment, the *Bam*HI end of which had been blunted by treatment with the Klenow fragment of *Escherichia coli* DNA polymerase I.

The fusion p163E.tk, which has the C-rich strand of the GC boxes reading in the sense of the *tk* mRNA (the "SV40 early-like" orientation), was made by insertion of the *Sph* I–*Ava* I fragment of p7.02 into pUC 18, cleavage with *Sph* I (position 63 in the monkey segment), digestion to nucleotide 163 with BAL-31, and insertion of a blunt-ended *Bam*HI fragment bearing the *tk* gene.

Binding and Transcription Reactions. Whole-cell extract from uninfected human (HeLa) cells (21) was fractionated by use of heparin-agarose, DEAE-Sepharose CL-6B, and Sephacryl S-300 gel-permeation chromatography (12). For DNase protection "footprinting" experiments, pooled Sephacryl fractions were used. For transcription, this material was further fractionated by phosphocellulose chromatography to separate Sp1 from Sp2 (12). Each DNase footprinting reaction (13, 22) contained 20 fmol of singly end-labeled probe and 1 μg of unlabeled sonicated calf thymus DNA carrier. *In vitro* transcription was carried out as described (12), using 250 ng of circular plasmid as template in each reaction. RNA synthesis was measured by the primer-extension method (13).

RESULTS

Binding of Sp1 to Monkey Genomic DNA. A double-stranded DNA probe for footprinting reactions was prepared by cleaving p7.02 DNA with *Ava* I (see map, Fig. 1), ^{32}P-labeling at 5′ ends with polynucleotide kinase, cleaving with *Sal* I, and isolating the end-labeled fragment by preparative gel electrophoresis. This probe DNA was incubated with various amounts of an Sp1-containing protein fraction (see *Materials and Methods*), subjected to limited cleavage with DNase I, and analyzed in an 8% polyacrylamide/urea sequencing gel (Fig. 1, *Ava* I 5′ probe). Two regions of protection became evident as the amount of Sp1 in the reaction was increased; these are labeled α and β. Examination of the Maxam–Gilbert sequence markers (23) on the right side of the panel shows that the α and β sites fall within regions containing multiple copies of the GC box, which on this strand reads "CCGCCC." The exact placement of the Sp1 protection sites in the monkey sequence is shown in Fig. 2. The Sp1-protected region of SV40 DNA (Fig. 1, SV40 *Eco*RI 5′ probe), which also contains GC boxes, has been aligned for comparison.

The binding results seen with the *Ava* I 5′ probe were confirmed by examining the pattern of binding on the opposite strand of the DNA. We prepared a separate probe by 5′ labeling the *Ava* I–*Sal* I fragment at the opposite end. As before, Maxam–Gilbert sequence markers were prepared. Protection in the β region was evident (Fig. 1, *Sal* I 5′ probe), but protection of the α region was not visible because these sequences were too far from the labeled end, and thus were compressed at the top of the gel. To circumvent this problem and examine the α region, we prepared a third probe by cutting p7.02 with *Ava* I, labeling at 3′ ends with avian myeloblastosis virus reverse transcriptase, and cutting again with *Sal* I (*Ava* I 3′ probe). Both α- and β-region protection was seen. These results were consistent with those seen initially, and are diagrammed in Fig. 2. Sp1 protects sequences in the same region on both strands of the DNA.

The overall size of the protected sequence was different in the two regions. The α region was smaller, with 14 bp of protection on one strand and 23 bp on the other, plus partial protection of a further 10 bp on each strand. The β region, in contrast, showed 54–55 bp of complete protection, with 8 bp more of partial protection on one strand, and was similar in size to the Sp1-binding region of SV40. There is some imprecision inherent in the footprinting technique, in part

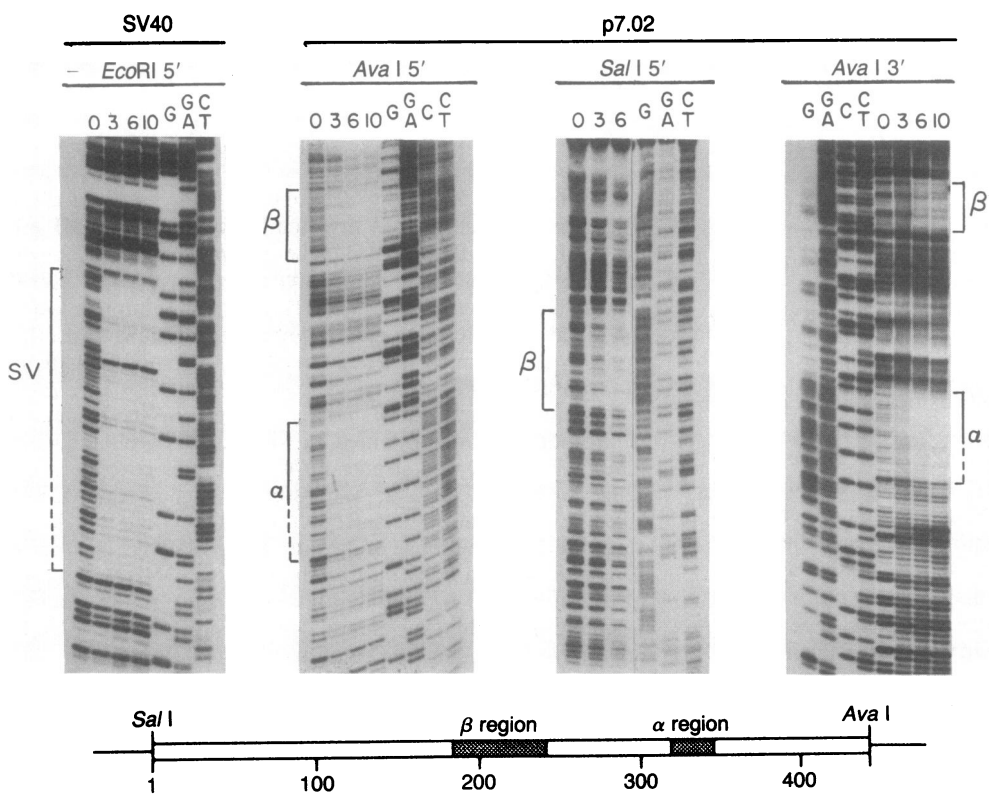

FIG. 1. DNase protection assay with SV40 and 7.02 probes. Reactions were carried out with 0, 3, 6, or 10 μl of Sp1-containing protein fraction present, as indicated at the tops of the lanes. Vertical brackets mark α and β protected regions (solid line, complete protection; dashed line, partial protection). DNA sequence markers were prepared from each probe (23) and are labeled as G, GA, C, or CT. Singly end-labeled probes were prepared from 7.02 DNA as described in the text and from SV40 DNA by labeling at an *Eco*RI site upstream of the early promoter (13).

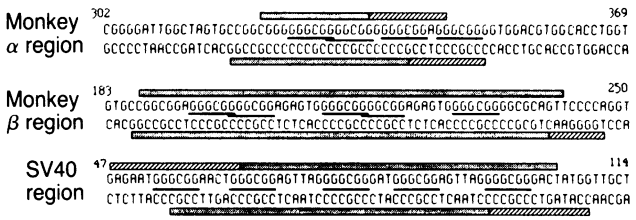

FIG. 2. Position of Sp1-protected regions within the monkey and SV40 DNA sequences. Data is taken from Fig. 1 and other similar results. Regions of complete and partial protection on each strand are shown as stippled and hatched bars, respectively. Heavy lines between strands indicate GGGCGG repeats. Numbering of monkey sequence is according to Queen *et al.* (16); numbering of SV40 sequence is according to Tooze (1).

because DNase I cleavage-site preferences lead to irregularities in the ladder of bands even without Sp1 present. However, it seems likely that the large difference observed between the α site and the other two sites must reflect a real difference in the size or conformation of the Sp1–DNA complex.

Competition Between SV40 and Monkey DNA for Sp1 Binding. The dominance of the GGGCGG sequence motif in both the SV40 and monkey binding regions, as well as the occurrence of binding activity for all three regions in the same protein fraction, suggests that it is the same molecule that is responsible for protection in each case. We confirmed this directly by using nonradioactive SV40 or monkey DNA to compete for binding to probe of the opposite type (Fig. 3). Both monkey and SV40 DNA were able to compete for binding to SV40 probe, and reciprocally, both SV40 and monkey DNA were able to suppress protection of the β region of the p7.02 AVA I 5′ probe. Results with the α region were similar (not shown).

Transcription of Monkey DNA. Although the identification

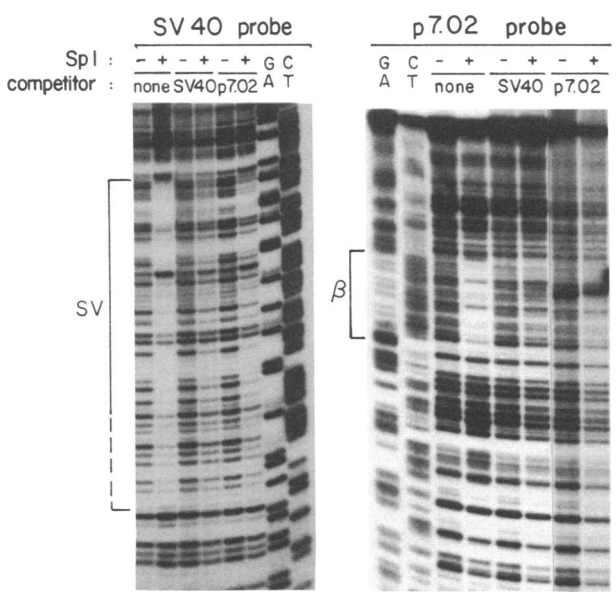

FIG. 3. Competition between SV40 and 7.02 DNA. (*Left*) Labeled SV40 probe (*Eco*RI 5′; see Fig. 1) was incubated in the presence of 2 μg of sonicated calf thymus DNA carrier and either unlabeled pSV07 DNA (a plasmid with three copies of the SV40 promoter region) or isolated *Ava* I–*Sal* I fragment from 7.02 DNA. When present, the competitor was in 15-fold molar excess over labeled probe. Reactions contained either no Sp1 or 6 μl of an Sp1-containing protein fraction. Maxam–Gilbert sequence markers are at right. (*Right*) Labeled 7.02 probe (*Ava* I 5′) incubated with Sp1 in the presence or absence of competitor under the same conditions. Maxam–Gilbert sequence markers are at left.

of Sp1 binding sites in 7.02 DNA suggested that Sp1 had a direct role in activating transcription of the promoter, it was important to confirm this hypothesis through *in vitro* transcription experiments. Previously, it was found that transcription of certain SV40 RNAs was highly dependent on Sp1, whereas other promoters that lacked GC boxes were transcribed at equal levels in the presence and absence of Sp1 (12). When 7.02 DNA was tested, some Sp1-dependent transcription was seen *in vitro*, using run-off transcription and nuclease S1 analysis, but the transcripts that were seen were not abundant; moreover, the major *in vivo* initiation sites at nucleotides 315 (late-like) and 103 (early-like) were not consistently represented among the *in vitro* products.

The 7.02 promoter does not contain the conserved sequence moiety known as the TATA box that is found 25–30 bp upstream of the transcriptional start site in many eukaryotic promoters (reviewed in ref. 24). The 7.02 promoter, like certain others, has evidently evolved a means of directing transcription in the absence of this sequence (17). Reasoning that the Sp1 binding sites of the 7.02 DNA might be functioning properly as a distal promoter element but that whatever functions as an alternative to the TATA box in 7.02 was inoperative in our *in vitro* system, we decided to fuse the Sp1-binding regions of monkey DNA to the TATA box of a foreign promoter. The herpes simplex virus *tk* promoter was a logical choice; like many eukaryotic promoters, its activity depends on both a proximal TATA box signal and a distal signal. By removing the distal signal, one creates an inactive promoter that can be reactivated by introducing a substitute promoter sequence in the upstream region. In the case of *tk*, a number of mutants were available in which portions of the promoter had been deleted and the remainder fused to synthetic linker DNA (19), creating molecules readily usable as fusion partners. We had already found that fusion of the SV40 GC boxes reactivated the truncated *tk* promoter (unpublished data), and we wished to see whether the monkey segments would do the same.

A map of the fusion clones is shown in Fig. 4, and details of their construction are given in *Materials and Methods*. One clone, p254L.tk, places the monkey binding region β very near the TATA box, with only about 52 bp of DNA separating the Sp1-protected region from the *tk* transcriptional start site. A second, p274L.tk, places the β region 72 bp from the start, and a third, p363L.tk, places the α region 66 bp from the start. The GC boxes in all three of these clones are present in the orientation resembling the SV40 late transcription direction, that is, with the G-rich strand reading in the same sense as the mRNA.

Activity of these constructs was tested by using them as templates for *in vitro* transcription (Fig. 5). The plasmid pSV01, containing the SV40 promoter region, was included as a positive control. Early-direction transcription of SV40, assayed here, was strongly dependent on Sp1. The plasmids pALP, containing the adenovirus-2 major late promoter, and ptk/Δ5′-32, containing the truncated *tk* promoter, were included as additional controls. The adenovirus promoter was transcribed at the same level with or without Sp1, and the truncated *tk* promoter was not transcribed at all.

The fusion construct p254L.tk was quite active as a template, and its transcription was as strongly dependent on Sp1 as that of SV40 (Fig. 5). The size of the extended product matched that expected for an RNA initiating at the normal wild-type *tk* initiation site. Somewhat unexpectedly the closely related construct p274L.tk, was substantially less active in transcription and showed less Sp1 dependence. This result indicates that the exact spacing of the GC box relative to the other parts of the promoter may be important in determining the level of transcription. A third construct, p363L.tk, showed an intermediate level of Sp1-dependent transcriptional activity, probably brought about by the prox-

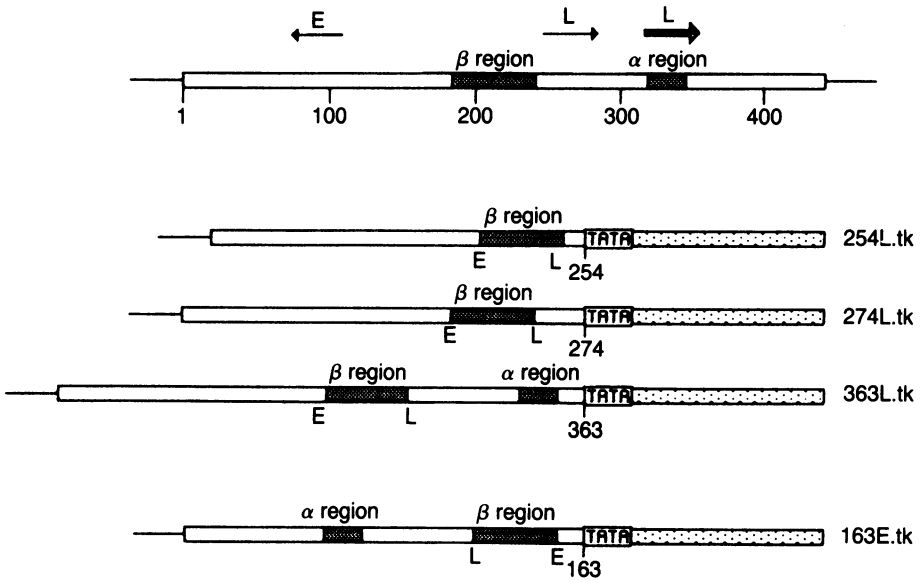

FIG. 4. Map of the 7.02 segment showing the α region, β region, and predominant *in vivo* transcripts, as detected by nuclease S1 analysis. The letters E and L above the arrows designate transcription in the early- and late-like directions, assigned according to homology with SV40. The numbers below the junction in the fusion clone maps indicate the position of the junction in the monkey sequence. Monkey DNA was joined to *tk* DNA via linkers (see *Materials and Methods*). The *tk* TATA box sequence, TATTAA is 21–26 bp upstream from the *tk* transcriptional start. Clones 254L.tk, 274L.tk, and 363L.tk were constructed to mimic transcription in the late-like direction; 163E.tk mimics transcription in the early-like direction.

imity of the α site, although we cannot rule out an effect of the more distant β site. The overall relationship between the point of fusion and Sp1-dependent transcriptional activity is summarized in Table 1. In interpreting this table, it should be kept in mind that the position of the bound molecule, as measured by DNase protection, is only approximate, and that comparison of the spacing in different promoters is subject to some inaccuracy. Nevertheless, it seems clear that the spacing is important, with larger distances between Sp1 and the TATA box tending to lead to lower levels of template activity in the *in vitro* reaction.

We were also interested in testing the ability of the monkey GC boxes to direct transcription in the early-like direction, with the C-rich strand reading in the sense of the mRNA. Despite the asymmetry of the GC box moieties, there was reason to think that they would function in both directions: the transcription maps of SV40 and monkey DNA show RNA complementary to both strands; moreover, the GC boxes of SV40 can be inverted and will retain promoter activity (6). We made a construct in which the β region was fused to the truncated *tk* gene in the early-like orientation, with the junction point 23 bp from the protected region. This template, p163E.tk, was less active than SV40 or p254L.tk, although Sp1-dependent transcription was seen in some experiments (Fig. 5). We do not know whether the greater activity with fusions in the late-like orientation was due to an intrinsic strand-preference of Sp1 bound at the β site or whether other factors, such as the spacing between the β site and the TATA box, also played a role. Our findings are consistent, however, with the observation that the 7.02 segment was one-fifth as active when inserted into an expression vector in the early-like orientation (17).

It is interesting to see that in this fusion, some Sp1-dependent transcripts originated upstream from the normal *tk* start near the junction between the monkey and *tk* DNA (Fig. 5, band near top of p163E.tk autoradiogram). Some starts at this site are faintly visible with other constructs as well, notably p274L.tk.

DISCUSSION

We have demonstrated that a factor from HeLa cells binds two regions in a monkey genomic promoter segment. Competition experiments, as well as direct comparison of the sequences within the bound regions, indicate this factor is

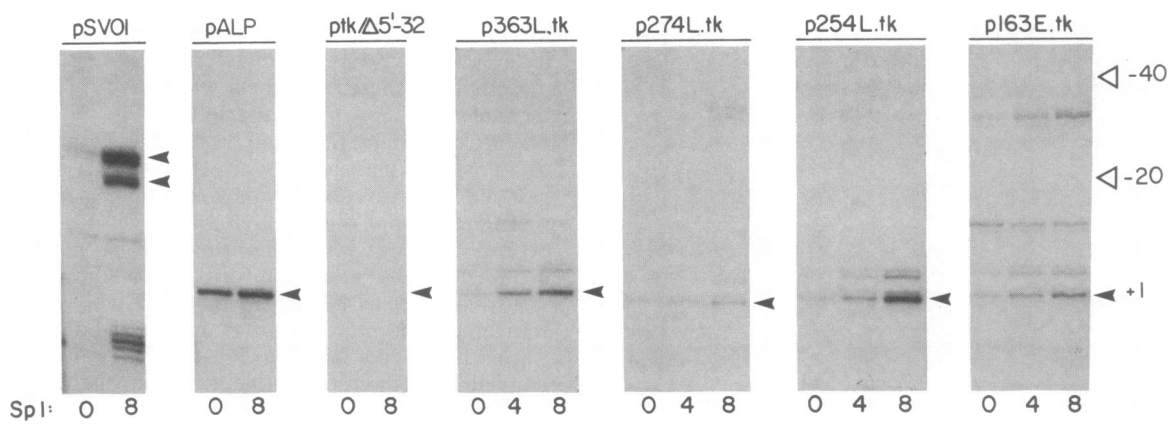

FIG. 5. Transcription of 7.02–*tk* fusion clones. *In vitro* transcription reactions contained endogenous RNA polymerase II; Sp2; and 0, 4, or 8 μl of an Sp1-containing fraction from a phosphocellulose column (see *Materials and Methods*). All transcripts were assayed by the primer-extension method. The plasmid pSV01 contains the SV40 early promoter, and transcripts from this template were hybridized to a natural SV40 primer as described (12). The plasmid pALP contains the adenovirus-2 major late promoter abutted to the *Pvu* II site of pBR322, and transcripts were hybridized to a 24-nucleotide synthetic primer that hybridizes just beyond the junction. Other templates are fusions to the *tk* promoter and are described in the text. RNAs from these templates were hybridized to a 24-nucleotide *tk*-specific synthetic primer. The scale at far right indicates map position of initiation sites in the *tk* gene, relative to the *in vivo* start at +1. All lanes are from the same experiment and were exposed for the same length of time. Autoradiography was without an intensifying screen, so that film response would be more nearly linear.

Table 1. Transcription of fusion clones

Clone	Sp1-binding region	Distance to start, bp	Transcription
p254L.tk	Monkey β	52	+++
p274L.tk	Monkey β	72	+/−
p363L.tk	Monkey α/β	66	++
p163E.tk	Monkey β/α	63	+/−
pSV01	SV40	63	+++

Distance is measured from the start site for RNA synthesis to the edge of the nearest Sp1-protected region. Boundaries of the protected regions are taken as nucleotides 186 and 242 for the monkey β site and nucleotide 345 for the monkey α site (see map, Fig. 2). The boundary of the SV40 region is taken as nucleotide 55 (for numbering, see ref. 1).

Sp1, previously identified as a host-specified transcription factor required for SV40 gene expression. Because the Sp1 binding sites from monkey DNA direct *in vitro* transcription when fused to a heterologous promoter, we believe that these sites are functional promoter elements and that Sp1 binding probably plays a role in the biological activity of the 7.02 DNA segment *in vivo*.

By nucleic acid hybridization analysis, the 7.02 segment appears to belong to an interspersed repetitive family with about 80 members (17). If other members of the family carry Sp1 binding sites, Sp1 may regulate a wide group of genes. Identification of gene products encoded by the regions of the monkey chromosome flanking the 7.02 segment, or information about changes in their level under different conditions, might allow us to deduce a possible role for Sp1 in the regulation of cellular gene expression. At present, our information is limited. Transcripts from the 7.02 region are known to be present in dividing tissue culture cells, where they were first reported (17). There is substantial single-copy DNA flanking the 7.02 segment (18), but the details of mRNA structure are yet to be elucidated, and no identification of gene products has been made.

In DNase-protection experiments, both the α and β regions appear as blocks of protection without distinguishing internal features. Previously, however, dimethyl sulfate-modification protection experiments (14) showed that the SV40 and 7.02 β regions each contain three well-separated clusters of nucleotide residues that make contact with Sp1. Each cluster lies within a GC box; moreover, contacts are limited to the major groove and fall on one strand of the DNA. The separate clusters of contacts, as well as the differences in size of the DNase-protected regions, support the idea that multiple protomers of Sp1 are bound to the SV40 and 7.02 β regions, and that fewer molecules of Sp1, perhaps only one, are bound to the smaller, α region.

One of the interesting features of Sp1-mediated transcription is that binding of the factors to an asymmetric DNA sequence allows activation of transcription in both directions. In the monkey promoter, the late-like, or GGGCGG, orientation of the binding site appears to be the more active, but in SV40 the amount of transcription in the two directions is under temporal regulation, with strand-switching occurring at the onset of viral DNA replication. It may be that the Sp1–DNA complex can assume different conformations as the result of sequences flanking the GC boxes or that in larger regions, multiple protomers of Sp1 can pack differently, bringing about a preference for one strand or the other. Transcription may also be influenced in a positive or negative way by proteins bound at neighboring sites, such as TATA boxes or the SV40 replication origin.

The enhancement of *in vitro* transcriptions seen when the Sp1 binding region is joined to elements of the *tk* promoter suggests that close placement of the TATA box facilitates interaction between Sp1 and other proteins that are required for transcriptional initiation. A similar result was seen when the SV40 GC boxes were fused to the *tk* TATA box (unpublished data). This facilitation of the interaction by proximal DNA sequences is clearly not so essential *in vivo*, where the major late-like transcript initiates both in the absence of a TATA box (17) and further from the monkey Sp1 binding region β than in the *tk* fusion constructs (73 bp vs. 52 bp, see Table 1). Similarly, early transcription of SV40 proceeds *in vivo* even when the TATA box is removed (3, 25), and late transcription, which is dependent on sequences in the Sp1 binding site, initiates 200 bp away and in the apparent absence of a TATA box (3, 5, 9). With the SV40 promoters, this *in vivo* behavior can be mimicked in unfractionated cell extracts (2, 4, 5, 8, 10, 11, 26–28) although not, as yet, in a fractionated system. Future experiments may clarify what conditions or additional factors are required for TATA box-independent *in vitro* transcription.

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1. Tooze, J. (1981) *Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), 2nd Ed., Part 2.
2. Myers, R. M., Rio, D. C., Robbins, A. K. & Tjian, R. (1981) *Cell* **25**, 373–384.
3. Fromm, M. & Berg, P. J. (1982) *J. Mol. Appl. Genet.* **1**, 457–481.
4. Lebowitz, P. & Ghosh, P. K. J. (1982) *J. Virol.* **41**, 449–461.
5. Hansen, U. & Sharp, P. A. (1983) *EMBO J.* **2**, 2293–2303.
6. Everett, R. D., Baty, D. & Chambon, P. (1983) *Nucleic Acids Res.* **11**, 2447–2464.
7. Baty, D., Saldaña-Barrera, H. A., Everett, R. D., Vigneron, M. & Chambon, P. (1984) *Nucleic Acids Res.* **12**, 915–932.
8. Brady J. N., Radonovich, M., Thoren, M., Das, G. & Salzman, N. P. (1984) *Mol. Cell. Biol.* **4**, 133–141.
9. Hartzell, S. W., Byrne, B. J. & Subramanian, K. N. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 23–27.
10. Mishoe, H., Brady, J. N., Radonovich, M. & Salzman, N. P. (1984) *Mol. Cell Biol.* **4**, 2911–2920.
11. Rio, D. & Tjian, R. (1984) *J. Mol. Appl. Genet.* **2**, 423–435.
12. Dynan, W. S. & Tjian, R. (1983) *Cell* **32**, 669–680.
13. Dynan, W. S. & Tjian, R. (1983) *Cell* **35**, 79–87.
14. Gidoni, D., Dynan, W. S. & Tjian, R. (1984) *Nature (London)* **312**, 409–413.
15. McCutchan, T. F. & Singer, M. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 95–99.
16. Queen, C., Lord, S. T., McCutchan, T. F. & Singer, M. F. (1981) *Mol. Cell. Biol.* **1**, 1061–1068.
17. Saffer, J. & Singer, M. F. (1984) *Nucleic Acids Res.* **12**, 4769–4788.
18. Lord, S. T. & Singer, M. F. (1984) *Mol. Cell. Biol.* **4**, 1635–1637.
19. McKnight, S. L., Gavis, E. R., Kingsbury, R. & Axel, R. (1981) *Cell* **25**, 385–398.
20. Vieira, J. & Messing, J. (1982) *Gene* **19**, 259–268.
21. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3855–3859.
22. Galas, D. & Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3170.
23. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–580.
24. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349–383.
25. Benoist, C. & Chambon, P. (1981) *Nature (London)* **290**, 304–310.
26. Rio, D. R., Robbins, A. K., Myers, R. M. & Tjian, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5706–5710.
27. Mathis, D. & Chambon, P. (1981) *Nature (London)* **290**, 310–315.
28. Vigneron, M., Barrera-Saldaña, H. A., Baty, D., Everett, R. G. & Chambon, P. (1984) *EMBO J.* **3**, 2373–2382.